REMARKS

Claims 1, 2, 9 and 23 have been amended to identify that the antimicrobial peptides and derivatives are selected from melittin, cecropin, and magainin or derivatives thereof. Support for these amendments can be found in the Specification for example at page 9, lines 5-6 and page 9, lines 13-17. The pre and prepro forms of melittin and cecropin were disclosed in Claim 3 as originally filed. Similarly, Claims 27, 28, 35 and 49 have been amended to identify that the antimicrobial peptides and derivatives are directed to a subset of the disclosed antimicrobial peptides selected from melittin, premelittin and prepromelittin.

Claim 12 has been amended to correct a minor grammatical error.

Claim 30 has been amended for proper claim dependency.

Claims 3, 24, 29, 32, 33, 41, 50 and 51 have been cancelled.

New Claims 53-77 have been added. Claims 53 and 54 are directed to methods of treatment of a subset of disorders disclosed in Claim 49. Claims 55-77 are directed to a subset of the disclosed antimicrobial peptides and derivatives, specifically cecropin, prececropin, preprocecropin, SB-37, Shiva-1, and derivatives thereof. Support for these claims can be found in the Specification, for example, at page 9, lines 5-6 and page 9, lines 13-17, and Claim 3 as originally filed.

Rejection of Claims 1-52 Under 35 U.S.C. §112, First Paragraph

Claims 1-52 stand rejected under 35 U.S.C. §112, first paragraph for the reasons advanced on pages 2-5 of the prior Office Action (paper No. 17). Specifically, the Examiner states that "[t]he claims recite '*therapeutic antimicrobial peptide or a biologically active derivative which is a part, analogue or homologue of the antimicrobial peptide*'. . . and . . . . as written encompass a large number of known or unknown antimicrobial peptides having antimicrobial property" (Office Action, page 2).

The Examiner refers Applicants to the Revised Interim Guidelines for the written description requirement (Office Action, page 3). The Examiner states that "melittin is only one species of the antimicrobial peptide family, which encompass a wide range of endogenously secreted peptides including lytic peptides and conventional antibiotics" (Office Action, page 3, last paragraph), and that "[t]he genus encompasses any peptide which inhibits the growth of a

microorganism secreted by any animal, thus encompassing an uncountable number of possible peptides" (Office Action, page 4).

The Examiner also states that "even within the species of melittin, significant variations concerning structure-functional relationships have been observed both in the teachings of those skilled in the art and exemplarily embodiment of the instant specification" (Office Action, page 4 second paragraph).

The Examiner again refers Applicants to the Revised Interim Guidelines for "Written Description" relating to possession of the invention (Office Action, page 4, last paragraph), and states that "the limited disclosure in the specification to melittin and its analog could not be extend [sic] to derivatives of *any* and *all* types of antimicrobial peptides, secreted by *any* and *all* animals which are less well studied, relatively unknown, or waiting to be discovered" (Office Action at page 5, third paragraph).

The MPEP (eighth edition) at §2163, subsection I, specifies that:

> An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. Possession may be shown in a variety of ways including description of an actual induction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention is complete, or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention (Citations omitted, emphasis added).

As amended, Applicants' claimed invention relates to a recombinant vector comprising retroviral vector DNA or a portion thereof necessary for infection and expression, and one or more coding sequences which encodes for a naturally occurring therapeutic antimicrobial peptide or a biologically active derivative thereof, wherein the antimicrobial peptide or derivative thereof is selected from the group consisting of melittin, premelittin, prepromelittin, cecropin, prececropin, preprocecropin, magainin, a part thereof, an analogue thereof, a homologue thereof and a combination thereof. The Examiner has stated in the Office Action at page 6, first sentence, that "the described SB-47, Shiva-1, and the described melittin analog meet the written description provision of 35 U.S.C. §112, first paragraph". In the Specification as filed,

Applicants have clearly described the claimed invention, particularly as amended. In addition, Applicants point out that new Claims 53, 54, 57 and 58 are directed to these specific analogues.

Applicants have clearly demonstrated possession of their invention. The antimicrobial peptides of Claim 1, and claims dependent thereon, are not *any* antimicrobial peptide. Melittin is the main component of honey bee toxin (Specification, page 2, line 15). Cecropins have been isolated from the pupae of the giant silk moth (Specification, page 3, line 13). Magainin was isolated from Xenopus laevis skin (Exhibit 1, Toxicology (1994) 87: 175-188). Thus, these antimicrobial peptides are not just *any* antimicrobial peptides that can be isolated from *any* or *all* animals.

In addition, Applicants have already asserted and established that methods of obtaining biologically active derivatives of the said antimicrobial peptides are well-known to those of skill in the art. Methods for assessing whether a particular portion of an antimicrobial peptide is biologically active were also know at the time of the Applicants invention, for example, see Perez-Paya *et al.,* 1995, under the heading "hemolytic and antimicrobial assays" . As previously stated by the Applicants, one of ordinary skill in the art can reasonably predict the substitution, deletion or addition of single amino acids from one of the ends of an antimicrobial peptide would likely produce a portion of an antimicrobial peptide that retains biological activity. Such modifications, which do not effect the microbial peptide, were known at the time of the Applicants' invention. As already stated in our previous reply, analogs of cecropin B which have as little as 40% sequence homology to the original cecropin B still remain functional (Specification, page 4, lines 7-14). At the time of the present invention, it was already known that cecropin analogues having the same charge distribution and hydrophobicity as an antimicrobial peptide retain biological activity. Applicants teach that melittin analogues are functional, specifically, for example, when the last six C-terminal amino acid residues are replaced by glycine (page 9, lines 24-30) and/or are comprise an amphophilic helix with or without signal peptide and activation domains (page 10, lines 18-20).

Furthermore, Applicants have provided specific descriptions of "distinguishing identifying characteristics" sufficient to demonstrate possession of the invention in the Specification. The anti-cancer (anti-tumour) activity of melittin, cecropins and magainins is known. For example, cecropin, prepromelittin and premelittin have been demonstrated as having anti-tumour effects, as disclosed in the Specification at page 25, lines 6- 26. Antiviral activity has also been specifically demonstrated for melittin, prepromelittin, premelittin, cecropin and

preprocecropin (Specification, page 25, line 27 - page 26, line28). Magainins are also known to be a related group of antimicrobial peptides (Specification, page 4, lines 15-16).

Clearly, Applicants have sufficiently described their invention with identifying characteristics to convey to one of ordinary skill in the art that the applicants were in possession of the invention. Withdrawal of the rejection is respectfully requested.

Rejection of Claims 1-26 Under 35 U.S.C. §112, First Paragraph

The Examiner maintains that "the new matter rejection advanced on page 6, 2nd and 3rd paragraph of paper #17" (Office Action, page 6).

Applicants believe that the Examiner refers to the new matter rejection on page 8, 2nd and 3rd paragraph of paper #17. Nevertheless, in light of the new claim amendments now presented, the rejection is obviated.

Rejection of Claims 1-52 Under 35 U.S.C. §112, First Paragraph

The prior enablement rejection of Claims 1-52 have been maintained by the Examiner for the reasons advanced in the Office Actions of paper number 17 and paper number 9. Specifically, the Examiner states that:

> [T]he specification, while being enabling for making an analog or homolog of melittin and cecropins having antimicrobial and antitumor activities *in vitro* and *in vivo* in an animal model, does not reasonably provide enablement for making and using analogs or homologues of *the genus of* antimicrobial peptides, treating any and all diseases selected from the group consisting of: a genetic defect, cancer and viral infections (Office Action at page 7, second whole paragraph).

The Examiner further states that:

> [T]he specification fails to provide sufficient guidance regarding how to make a partial or combination, an analogue or homologue for any and all AMPs, whether the specific embodiment of the invention would apply to any and all antimicrobial peptides, what are the structures of these peptides and what structural change it would tolerate so that the derivatives would still be capable of killing microorganisms" (Office Action at pages 7-8, bridging paragraph).

Applicants point out that the claims, as amended, relate to a recombinant vector comprising retroviral vector DNA or a portion thereof necessary for infection and expression, and one or more coding sequences which encodes for a naturally occurring therapeutic antimicrobial peptide or a biologically active derivative thereof, wherein the antimicrobial peptide or derivative thereof is selected from the group consisting of melittin, premelittin, prepromelittin, cecropin, prececropin, preprocecropin, magainin, a part thereof, an analogue thereof, a homologue thereof and a combination thereof. Thus, the claims do not encompass *any* and *all* antimicrobial peptides. There would be no undue experimentation required by one of skill in the art to practice the claimed invention, particularly as amended.

Derivatives, Analogues And Homologues Are Recognized By One Of Skill In The Art

As previously stated derivatives, analogues and homologues that have antimicrobial activity would be readily obtained by one of skill in the art. The sequences of magainin, cecropin and melittin were known to the person of ordinary skill in the art (see for example, Specification at page 9, lines 22-23). It was known at the time the invention was made how to make derivatives of melittin, cecropin and magainin. In particular, it was known how deletion variants, substitution variants, and variants with added amino acids can be made. Moreover, it was common general knowledge that conservative amino acid substitutions do not usually have a detrimental effect on the function of a peptide or a protein. Thus, the person skilled in the art could even have predicted which variants would probably have biological activity.

Furthermore, the application discloses biological tests allowing for the determination of whether a derivative of melittin, cecropin or magainin has the desired biological activity (see pages 25-27 of the Specification). Moreover, the application discloses specific examples of derivatives according to the present invention. The Specification at page 4, first full paragraph, discloses Shiva-1, which is a biologically active cecropin B analogue sharing only 40% sequence homology, indicating that even extensive mutations of the claimed peptides do not destroy biological activity. Another melittin analogue is disclosed on page 9, lines 28-29 of the Specification. This melittin analog has six out of twenty-six amino acids replaced, leaving 77% homology, while maintaining the analogues therapeutic benefit (see, for example, the Specification at page 9, line 26).

Magainin sequences are known, and are disclosed in the Specification as being a related group of antimicrobial peptides (page 4, lines 15-16). Thus, one of ordinary skill in the art will appreciate how to make magainin derivatives.

In summary, at the time of Applicants' invention, the person of ordinary skill in the art knew how to make derivatives of melittin, cecropin and magainin, and how to predict which derivatives will have biological activity. Furthermore, it was known at the time the invention was made, that extensive sequence variations could be made without destroying the biological function of the antimicrobial peptide.

The Claims Are Not Solely Directed To Gene Therapy

The Examiner further states that the claims encompass gene therapy and as such, the Specification fails to meet the enablement requirement (Office Action pages 9-10). Applicants point out that the recombinant vectors of Claim 1, and claims dependent thereon, can be used in a variety of experimental procedures. The broadest reasonable interpretation of this claim does not need to be restricted to gene therapy. Applicants have clearly stated that the invention provides for non-therapeutical methods for introducing homologous and/or heterologous nucleotide sequences into human or animal cells *in vitro* and *in vivo* (Specification at page 17, lines 18-21). Thus, the Examiners interpretation of the claimed invention is not the only reasonable interpretation of the claims as set forth.

The Specification Is Enabling For Gene Therapy To One Of Skill In The Art

Even in the context of therapeutical applications of the claimed invention, the general feasibility of gene therapy is well accepted in the art, and it is universally accepted by those of skill in the art. For example, Blaese, *et al.* (Exhibit 2: Science (1995) 270: 475-480) disclose a clinical trial involving retroviral-mediated transfer of the adenosine (ADA) gene into the T-cells of two children with severe combined immune deficiency (ADA-SCID). In this example of gene therapy, upon treatment, the number of T-cells normalized as did many cellular and humoral immune responses. In a further example, Bordignon *et al.* (Exhibit 3: Science (1995) 270:470-475) also used a retroviral vector for the same purpose as Blaese *et al.*, and came to similar results. In another example, Grossman *et al.* (Exhibit 4: Nature Genetics (1994) 6:335-341) report successful *ex vivo* gene therapy directed to the liver of a patient with familial hypercholesterolaemia.

Applicants have demonstrated that the anti-cancer activity of melittin, cecropins and magainins is known, see Specification, for example, at page 3, line 21 - page 4, line 18. Particularly, cecropin, prepromelittin and premelittin have been demonstrated by the Applicants as having anti-tumour effects (Specification at page 25, lines 6- 26). Antiviral activity was specifically demonstrated by the Applicants for melittin, prepromelittin, premelittin, cecropin and preprocecropin (Specification, page 25, line 27 - page 26, line28).

Thus, in light of the Specification as provided, with working examples demonstrating that administration of a recombinant vector comprising a sequence which encodes for an antimicrobial peptide successfully produces antitumor effects *in vivo* and antiviral effects *in vitro*, in addition to what was known to one of ordinary skill in the art as indicated in Exhibits 2-4, the Specification is clearly demonstrated as fully enabled for gene therapy. Notwithstanding, the Specification is enabling for non-gene therapy applications, therefore withdrawal of the rejection is respectfully requested.

Attachment For Form PTO-948

An attachment for PTO-948 entitled "Information on How to Effect Drawing Changes" was received with the Office Action, however, PTO Form 948 was not received by the Applicants. Thus, it is assumed that drawing corrections are not required at this time.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (978) 341-0036.

Respectfully submitted,
HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Anne J. Collins
Anne J. Collins
Registration No. 40,564
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
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MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Thrice Amended) A recombinant vector comprising, in operable linkage,
   a) retroviral vector DNA or at least a portion of the retroviral vector DNA comprising elements necessary for infection and direction of expression in target cells; and
   b) one or more coding sequences wherein at least one sequence encodes for a naturally occurring therapeutic antimicrobial peptide or a biologically active derivative [which is a part, analogue or homologue of the antimicrobial peptide and wherein the antimicrobial peptide is not defensin] thereof, wherein the antimicrobial peptide or derivative thereof is selected from the group consisting of: melittin, premelittin, prepromelittin, cecropin, prececropin, preprocecropin, magainin, a part thereof, an analogue thereof, a homologue thereof and a combination thereof.

2. (Thrice Amended) The recombinant vector according to Claim 1 comprising in operable linkage,
   a) a 5' long terminal repeat region comprising the structure U3-R-U5;
   b) one or more of said coding sequences wherein at least one sequence encodes for a naturally occurring therapeutic antimicrobial peptide or a biologically active derivative [which is a part, analogue or homologue of the antimicrobial peptide and wherein the antimicrobial peptide is not defensin] thereof, wherein the antimicrobial peptide or derivative thereof is selected from the group consisting of: melittin, premelittin, prepromelittin, cecropin, prececropin, preprocecropin, magainin, a part thereof, an analogue thereof, a homologue thereof and a combination thereof; and
   c) a 3' long terminal repeat region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a polylinker sequence, followed by the R and U5 region to undergo promoter conversion.

9. (Thrice Amended) A recombinant retroviral vector system comprising:

a) a recombinant vector comprising, in operable linkage,

i) retroviral vector DNA or at least a portion of the retroviral vector DNA comprising elements necessary for infection and direction of expression in target cells; and

ii) one or more coding sequences wherein at least one sequence encodes for [at least one] a naturally occurring therapeutic antimicrobial peptide or a biologically active derivative [which is a part, analogue or homologue of the antimicrobial peptide and wherein the antimicrobial peptide is not defensin] thereof, wherein the antimicrobial peptide or derivative thereof is selected from the group consisting of: melittin, premelittin, prepromelittin, cecropin, prececropin, preprocecropin, magainin, a part thereof, an analogue thereof, a homologue thereof and a combination thereof; and

b) a packaging cell line harboring at least one retroviral construct coding for proteins required for said retroviral vector to be packaged.

12. (Amended) A retroviral provirus produced by infection of target cells with a recombinant retroviral particle according to Claim 11 whereby the U3 sequence is duplicated during the process of reverse transcription in the infected target cell and appears in the 5' long terminal repeat and the 3' long terminal repeat of the resulting provirus, and the U5 of the 5' long terminal repeat is duplicated during the process of reverse transcription in the infected target cell and appears in the 3' long terminal repeat and in the 5' long terminal repeat of the resulting provirus.

23. (Twice Amended) A method for the treatment of a disease selected from the group consisting of: a genetic defect, cancer and viral infections, comprising administering to a subject in need thereof a therapeutically effective amount of a recombinant retroviral particle produced by transfecting a packaging cell line harboring at least one retroviral or recombinant retroviral construct coding for proteins required for said retroviral vector to be packaged, with a recombinant retroviral vector comprising, in operable linkage,

a) retroviral vector DNA or at least a portion of the retroviral vector DNA comprising elements necessary for infection and direction of expression in target cells; and

b) one or more coding sequences wherein at least one sequence encodes for a naturally occurring therapeutic antimicrobial peptide or a biologically active derivative [which is a part, analogue or homologue of the antimicrobial peptide and wherein the antimicrobial peptide is not defensin] thereof, wherein the antimicrobial peptide or derivative thereof is selected from the group consisting of: melittin, premelittin, prepromelittin, cecropin, prececropin, preprocecropin, magainin, a part thereof, an analogue thereof, a homologue thereof and a combination thereof.

27. (Amended) A recombinant vector comprising, in operable linkage,

a) retroviral vector DNA or at least a portion of the retroviral vector DNA comprising elements necessary for infection and direction of expression in target cells; and

b) one or more coding sequences wherein at least one sequence encodes for a naturally occurring therapeutic antimicrobial peptide or a biologically active derivative [which is a part, analogue or homologue of the antimicrobial peptide and wherein the antimicrobial peptide is an anti-retroviral peptide and/or an anti-tumor peptide] thereof, wherein the antimicrobial peptide or derivative thereof is selected from the group consisting of: melittin, premelittin, prepromelittin, a part thereof, an analogue thereof, a homologue thereof, and a combination thereof.

28. (Amended) The recombinant vector according to Claim 27 comprising in operable linkage,

a) a 5' long terminal repeat region comprising the structure U3-R-U5;

b) one or more of said coding sequences wherein at least one sequence encodes for a naturally occurring therapeutic antimicrobial peptide or a biologically active derivative [which is a part, analogue or homologue of the antimicrobial peptide and wherein the antimicrobial peptide is an anti-retroviral peptide and/or an anti-tumor peptide] thereof, wherein the antimicrobial peptide or derivative thereof is

selected from the group consisting of: melittin, premelittin, prepromelittin, a part thereof, an analogue thereof, a homologue thereof, and a combination thereof; and

c) a 3' long terminal repeat region comprising a completely or partially deleted U3 region wherein said deleted U3 region is replaced by a polylinker sequence, followed by the R and U5 region to undergo promoter conversion.

30. (Amended) The recombinant vector according to Claim [29] 28, wherein said polylinker sequence comprises at least one unique restriction site and, optionally, at least one insertion of a heterologous DNA fragment.

35. (Amended) A recombinant retroviral vector system comprising:

a) a recombinant vector comprising, in operable linkage,

i) retroviral vector DNA or at least a portion of the retroviral vector DNA comprising elements necessary for infection and direction of expression in target cells; and

ii) one or more coding sequences wherein at least one sequence encodes for at least one naturally occurring therapeutic antimicrobial peptide or a biologically active derivative thereof [which is a part, analogue or homologue of the antimicrobial peptide and wherein the antimicrobial peptide is an anti-retroviral peptide and/or an anti-tumor peptide] wherein the antimicrobial peptide or derivative thereof is selected from the group consisting of: melittin, premelittin, prepromelittin, a part thereof, an analogue thereof, a homologue thereof, and a combination thereof; and

b) a packaging cell line harboring at least one retroviral construct coding for proteins required for said retroviral vector to be packaged.

49. (Amended) A method for the treatment of a disease selected from the group consisting of: a genetic defect, cancer and retroviral infections, comprising administering to a subject in need thereof a therapeutically effective amount of a recombinant retroviral particle produced by transfecting a packaging cell line harboring at least one retroviral or recombinant retroviral construct coding for proteins required for said retroviral vector to be packaged, with a recombinant retroviral vector comprising, in operable linkage,

a) retroviral vector DNA or at least a portion of the retroviral vector DNA comprising elements necessary for infection and direction of expression in target cells; and

b) one or more coding sequences wherein at least one sequence encodes for a naturally occurring therapeutic antimicrobial peptide or a biologically active derivative [which is a part, analogue or homologue of the antimicrobial peptide and wherein the antimicrobial peptide is an anti-retroviral peptide and/or an anti-tumor peptide] thereof, wherein the antimicrobial peptide or derivative thereof is selected from the group consisting of: melittin, premelittin, prepromelittin, a part thereof, an analogue thereof, a homologue thereof, and a combination thereof.